USER GUIDE

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PureLink® Quick Gel Extraction Kit

For purifying DNA fragments from agarose gels

Catalog Numbers K2100-12, K2100-25

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Contents

Kit Contents and Storage

Intended Use

For research use only Not intended for any animal or human therapeutic or diagnostic use.

Types of Kits

This manual is supplied with the following kits:

Product	Cat. no.	Quantity
PureLink® Quick Gel Extraction Kit	K2100-12	50 reactions
	K2100-25	250 reactions

Shipping and Storage

All components of the PureLink® Quick Gel Extraction Kit are shipped at room temperature. Upon receipt, store all components at room temperature.

Contents

The components included in the PureLink® Quick Gel Extraction Kit are listed below. Sufficient reagents are included to perform 50 reactions for Cat. no. K2100–12 and 250 reactions for Cat. no. K2100–25.

Component	Quantity	
	Cat. no. K2100-12	Cat. no. K2100-25
Gel Solubilization Buffer (L3)	2 × 90 mL	10 × 90 mL
Wash Buffer (W1)	16 mL	5 × 16 mL
Elution Buffer (E5) (10 mM Tris-HCl, pH 8.5)	15 mL	5 × 15 mL
Quick Gel Extraction Columns in Wash Tubes	50 each	5 × 50 each
Recovery Tubes	50 each	5 × 50 each

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Important

The buffers included with this PureLink® Quick Gel Extraction Kit are reformulated to achieve optimal results. See below for a description of the buffer changes.

Note: Do not mix the buffers from this kit with any buffers from previous PureLink® Gel Extraction Kits because they are not compatible.

- Gel Solubilization Buffer (L3) is reformulated to allow faster melting of your gels.
- Wash Buffer (W1) is reformulated for better performance.
- Elution Buffer (E5) is reformulated for greater compatibility with enzymatic reactions.

Introduction

Overview

Introduction

The PureLink® Quick Gel Extraction Kit allows you to rapidly and efficiently purify DNA fragments from TAE or TBE agarose gels of various percentages. DNA can be extracted and purified from agarose gels with different melting points in ~30 minutes using PureLink® silica membrane-based Quick Gel Extraction Columns. For your convenience, purification protocols are provided for centrifugation and for vacuum.

Note: The PureLink® Quick Gel Extraction Kit is **not** designed to purify supercoiled plasmid DNA or genomic DNA from agarose gels. **Only linear DNA fragments may be purified from gels using these kits.**

Advantages

The advantages of using PureLink® Quick Gel Extraction Kit are the capabilities to:

- Purify DNA fragments from TAE and TBE agarose gels of various percentages and melting points
- Complete the procedure in ~30 minutes
- Easily purify DNA fragments from 40 bp to 10 kb from gels
- Obtain high recovery of DNA fragments
- Bind and purify up to 15 µg DNA with one column
- Purify DNA fragments that are high-quality and show reliable performance in PCR, restriction enzyme digestion, cloning, and labeling

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System Overview

To purify your desired DNA fragment from agarose gel using the PureLink® Quick Gel Extraction Kit, you will first excise the gel piece using the Gel Solubilization Buffer. The Gel Solubilization Buffer enables efficient extraction of the DNA fragment from TAE or TBE agarose gels without any additional solutions or modifications to the protocol. After the gel is sufficiently dissolved, you can purify and elute the DNA fragment using centrifugation or vacuum, as described below.

You will place the dissolved gel piece into a Quick Gel Extraction Column containing a silica membrane. The DNA will bind the membrane using centrifugation or vacuum. Then, you will wash the membrane with Wash Buffer containing ethanol to remove impurities and elute the purified DNA into a Recovery Tube using Elution Buffer (10 mM Tris-HCl, pH 8.5). The purified DNA is suitable for use in a wide variety of downstream applications.

Downstream Applications

The purified DNA is suitable for various downstream applications, including:

- DNA sequencing
- Cloning
- Restriction enzyme digestion
- PCR reactions
- Labeling

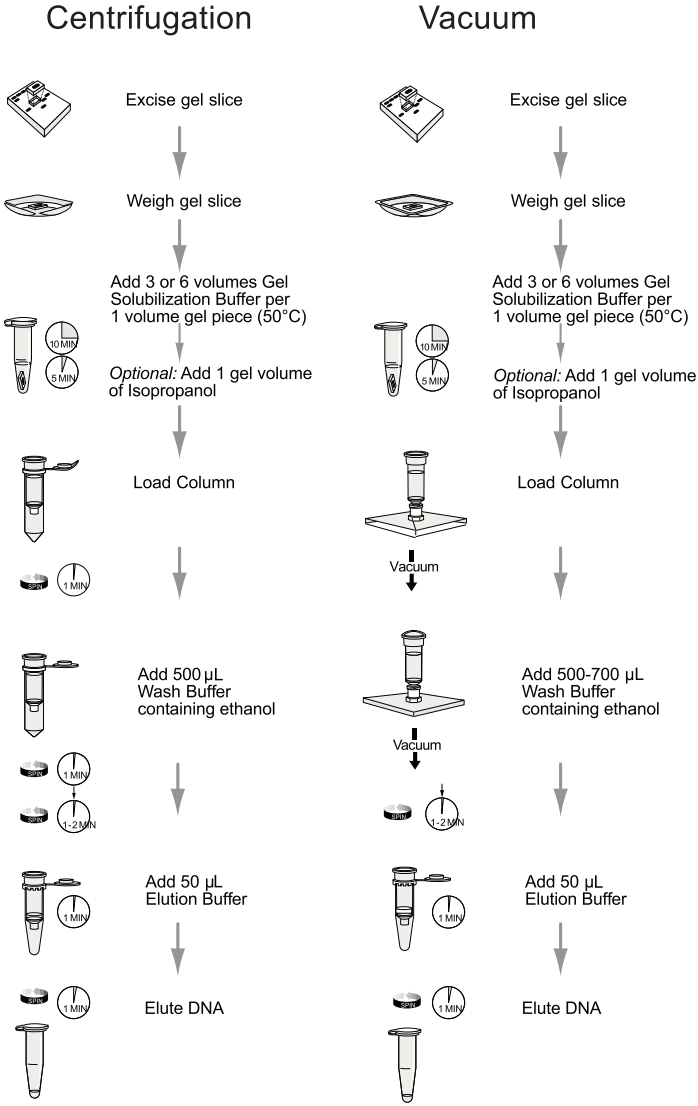
Kit Specifications

Starting Material:	≤400 mg agarose gel slice
Binding Capacity:	~15 µg DNA per column
Column Reservoir Capacity:	850 µL
Wash Tube Capacity:	2.0 mL
Recovery Tube Capacity	1.5 mL–1.7 mL
Centrifuge Compatibility:	>12,000 × g
Elution Volume:	50 µL
DNA Recovery:	Up to 95% (dependant on DNA fragment size)
DNA Fragment Size:	40 bp to 10 kb

Experimental Overview

Gel Extraction Purification Workflow

The flow chart below provides an overview for purifying DNA fragments from agarose gels using the PureLink® Quick Gel Extraction Kit.



Methods

Preparing the Gel Slice

Introduction

Instructions for preparing the gel slice containing the DNA fragment of interest are included in this section.

Note

The PureLink® Quick Gel Extraction Kit is **not** designed to purify supercoiled plasmid DNA or genomic DNA from agarose gels. **Only linear DNA fragments may be purified from gels using these kits.**

Materials Needed

- Agarose gel containing the DNA fragment
- Weighing paper or weigh trays
- Scale (sensitive to 0.001 g)
- Water bath or heat block set at 50°C
- 1.5-mL or 5 mL-polypropylene microcentrifuge tubes
- Clean, sharp razor blade
- *Optional*: 100% isopropanol

Component supplied with the kit:

- Gel Solubilization Buffer (L3)

Agarose Gels

The PureLink® Quick Gel Extraction Kit is suitable for purifying DNA fragments from various percentage TAE and TBE agarose gels and from agarose gels with different melting points, without modifying the protocol. **Follow specific directions regarding gels containing >2% agarose.**

DNA Fragments

- The PureLink® Quick Gel Extraction Kit is suitable for purifying DNA fragments from 40 bp to 10 kb in size. You may purify larger DNA fragments from gels using these kits; however, this may result in lower DNA recovery.
- Ensure that the DNA fragment of interest is **completely** separated from other DNA fragments on the agarose gel.
- Each Quick Gel Extraction Column can purify **up to 15 µg** of DNA. If you wish to purify a larger amount of DNA, use several Quick Gel Extraction Columns. For best results, use one Quick Gel Extraction Column per 10 µg of DNA fragment loaded onto the gel.

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- The Gel Solubilization Buffer (L3) contains guanidine isothiocyanate (an irritant). This chemical is harmful when in contact with the skin, or when it is inhaled or ingested.
- **Do not** add bleach or acidic solutions directly to solutions or sample preparation waste that contains guanidine isothiocyanate, as reactive compounds and toxic gases are formed.

For your protection, always wear a laboratory coat, gloves, and safety glasses when handling this chemical. Dispose of the buffer and chemicals in appropriate waste containers.

Follow the recommendations below to obtain the best results:

- To minimize DNA degradation, always wear gloves and use a clean razor blade to cut the gel slice.
- Maintain a sterile working environment when handling DNA to avoid any contamination from DNases.
- Ensure that no DNase is introduced into the sterile solutions supplied with the kit.
- Make sure all equipment that comes in contact with DNA is sterile, including pipette tips and tubes.

Preparing the Gel Slice

After completing agarose gel electrophoresis:

1. Excise the area of the gel containing your desired DNA fragment using a clean, sharp razor blade. Minimize the amount of agarose surrounding the DNA fragment.
2. Weigh the gel slice containing the DNA fragment using a scale sensitive to 0.001 g, then place the gel into a 1.5- or 5.0-mL microcentrifuge tube as described on page 6.

   Note: The maximum amount of starting material (gel) is ≤400 mg per tube. If your gel slice exceeds 400 mg, cut the gel into smaller slices so that no one piece exceeds 400 mg. Place each additional gel slice created into separate microcentrifuge tubes.
3. Proceed to **Dissolving the Gel**, page 6.

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Preparing the Gel Slice, Continued

Dissolving the Gel

1. **For ≤2% agarose gels:**

   - Place up to 400 mg of the excised gel containing the DNA fragment (previous page) into a **1.5-mL** polypropylene microcentrifuge tube.
   - Add 3 volumes Gel Solubilization Buffer (L3) for every 1 volume of gel (e.g., add 1.2 mL Gel Solubilization Buffer for a 400 mg gel piece).

   For >2% agarose gels:

   - Place up to 400 mg of the excised gel containing the DNA fragment (page 5) into a **5-mL** polypropylene microcentrifuge tube.
   - Add 6 volumes Gel Solubilization Buffer (L3) for every 1 volume of gel (e.g., add 2.4 mL for a 400 mg gel piece).

2. Place the tube(s) containing your gel slice and Gel Solubilization Buffer (step 1 of this procedure) into a 50°C water bath or heat block.
3. Incubate the gel slice and Gel Solubilization Buffer at 50°C for at least 10 minutes. Invert the tube by hand every 3 minutes to mix and ensure gel dissolution.

   Note: High concentration gels (>2% agarose) or large gel slices may take longer than 10 minutes to dissolve.
4. After the gel slice appears dissolved, incubate the tube for an **additional** 5 minutes.
5. *Optional*: For optimal DNA yields, add 1 gel volume isopropanol to the dissolved gel slice. Mix well.
6. Proceed to **Purifying DNA Using a Centrifuge** (page 7), or **Purifying DNA Using a Vacuum** (page 9).

Purifying DNA Using a Centrifuge

Introduction

This procedure is designed for purifying DNA fragments in approximately 30 minutes using a centrifuge.

Materials Needed

You will need the following items:

- 96–100% ethanol
- Microcentrifuge capable of centrifuging at >12,000 × g
- DNase-free pipettes and tips

Components supplied with the kit:

- Wash Buffer (W1)
- Elution Buffer (E5)
- Quick Gel Extraction Columns in Wash Tubes
- Recovery Tubes

Preparing Wash Buffer (W1) with Ethanol

Before beginning, prepare the Wash Buffer (W1) with ethanol as follows:

For K2100-12 (50 preps):

1. Add 64 mL 96–100% ethanol to the Wash Buffer.
2. Check the box on the Wash Buffer label to indicate that ethanol was added.
3. Store the Wash Buffer with ethanol at room temperature.

For K2100-25 (250 preps):

1. Add 64 mL 96–100% ethanol to **each** Wash Buffer bottle (5 Wash Buffer bottles are provided in this kit).
2. Check the box on the Wash Buffer labels to indicate that ethanol was added.
3. Store the Wash Buffer with ethanol at room temperature.

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Binding, Washing and Eluting DNA

Before beginning, add ethanol to the Wash Buffer (W1, see page 7).

1. Pipet the dissolved gel piece containing the DNA fragment of interest (steps 4–5, page 6) onto the center of a Quick Gel Extraction Column inside a Wash Tube.

   Note: Do **not** load >400 mg dissolved agarose per Quick Gel Extraction Column.

2. Centrifuge at >12,000 × g for 1 minute.
   Discard the flow-through and replace the Quick Gel Extraction Column into the Wash Tube.
3. Add 500 µL Wash Buffer (W1), containing ethanol (page 7) to the Quick Gel Extraction Column.
4. Centrifuge at >12,000 × g for 1 minute.
   Discard the flow-through and replace the column into the Wash Tube.
5. Centrifuge the column again at maximum speed for 1–2 minutes to remove any residual Wash Buffer and ethanol. Discard the Wash Tube and place the Quick Gel Extraction Column into a Recovery Tube.
6. Add 50 µL Elution Buffer (E5) to the center of the Quick Gel Extraction Column.
7. Incubate the column for 1 minute at room temperature.
8. Centrifuge the Column at >12,000 × g for 1 minute.
   The Recovery Tube contains the purified DNA.
   Discard the Quick Gel Extraction Column.
9. Store the purified DNA (see **Storing the Purified DNA**), or proceed to your downstream application of choice.

Storing the Purified DNA

Store the purified DNA at 4°C for immediate use or aliquot the DNA and store at –20°C for long-term storage.
Avoid repeated freezing and thawing of the DNA.

Purifying DNA Using a Vacuum

Introduction

This procedure is designed for purifying DNA fragments in approximately 30 minutes using a vacuum manifold.

Materials Needed

You will need the following items:

- 96–100% ethanol
- Vacuum manifold
- DNase-free pipettes and tips

Components supplied with the kit:

- Wash Buffer (W1)
- Elution Buffer (E5)
- Quick Gel Extraction Columns
- Wash Tubes
- Recovery Tubes

Preparing Wash Buffer (W1) with Ethanol

Before beginning, prepare the Wash Buffer (W1) with ethanol as follows:

For K2100-12 (50 preps):

1. Add 64 mL 96–100% ethanol to the Wash Buffer.
2. Check the box on the Wash Buffer label to indicate that ethanol was added.
3. Store the Wash Buffer with ethanol at room temperature.

For K2100-25 (250 preps):

1. Add 64 mL 96–100% ethanol to **each** Wash Buffer bottle (5 Wash Buffer bottles are provided in this kit).
2. Check the box on the Wash Buffer labels to indicate that ethanol was added.
3. Store the Wash Buffer with ethanol at room temperature.

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Binding, Washing and Eluting DNA

Before beginning, add ethanol to the Wash Buffer (W1, see page 9).

Perform all vacuum operations at room temperature.

1. Assemble the vacuum manifold according to the manufacturer's instructions.
2. Attach a Quick Gel Extraction Column to the vacuum manifold.
3. Pipet the dissolved gel piece containing the DNA fragment of interest (steps 4–5, page 6) onto the center of the silica membrane of the Quick Gel Extraction Column. Apply the vacuum until all of the liquid passes through the Column, then switch off the vacuum source.

   Note: Do not load more than 400 mg dissolved agarose per Column.
4. Add 500–700 µL Wash Buffer (W1) containing ethanol (page 9) to the center of the Column.
5. Apply the vacuum until all of the liquid passes through the Column, then switch off the vacuum. Remove the Quick Gel Extraction Column from the vacuum and place it into a Wash Tube.
6. Centrifuge the column with the Wash Tube at maximum speed for 1–2 minutes to remove any residual Wash Buffer and ethanol. Discard the flow-through **and** the Wash Tube. Place the Quick Gel Extraction Column into a Recovery Tube.
7. Add 50 µL Elution Buffer (E5) to the center of the Quick Gel Extraction Column.
8. Incubate the Column for 1 minute at room temperature.
9. Centrifuge the Column in the Recovery Tube at >12,000 × *g* for 1 minute to elute the purified DNA *into the Recovery Tube.* Discard the column.
10. Store the purified DNA (see **Storing DNA**), or proceed to your downstream application of choice.

Storing DNA

Store the purified DNA at 4°C for immediate use or aliquot the DNA and store at –20°C for long-term storage. Avoid repeated freezing and thawing of DNA.

Appendix

Estimating DNA Yield and Quality

Introduction

After purifying the DNA, you may determine the quantity and quality of the purified DNA.

DNA Yield

Measure DNA concentration using Qubit® DNA Assay Kits or UV absorbance at 260 nm.

Qubit® DNA Assay Kits

The Qubit® DNA Assay Kits (see page 13 for ordering information) provide a rapid, sensitive, and specific method for measuring dsDNA concentration with minimal interference from RNA, protein, ssDNA (primers), or other common contaminants that affect UV absorbance.

The kit contains a state-of-the-art quantitation reagent, pre-diluted standards for standard curve, and a pre-made buffer. For optimal results, perform the quantitation using a Qubit® 2.0 Fluorometer (see page 13). You can also use standard fluorescent microplate readers. Follow manufacturer's recommendations to perform the assay.

Estimating DNA Quality

Typically, DNA isolated using the PureLink® Quick Gel Extraction Kit has an A_{260}/A_{280} >1.8 when samples are diluted in Tris-HCl pH 7.5–8.5 indicating that the DNA is substantially free of contaminants that would otherwise affect UV absorbance.

To confirm the integrity and the size of the purified DNA fragment, perform agarose gel electrophoresis.

Expected Results

DNA fragments of various sizes were purified using PureLink® Quick Gel Extraction Kit as described in this manual. The concentration of purified DNA was measured with Qubit® DNA Assay Kits (see page 13 for ordering information). The DNA yields are listed in the table below.

DNA Size	Amount Loaded	% DNA Yield*
400 bp	0.5 µg	>85%
740 bp	1 µg	>85%
2.9 kb	1 µg	>85%

***Note:** The DNA yield varies with the fragment size, amount of DNA loaded on the gel, gel slice size, elution volume, and incubation time.

Troubleshooting

Introduction

Review the information below to troubleshoot your experiments with PureLink® Quick Gel Extraction Kit.

Observation	Cause	Solution
Low DNA yield	Incorrect ratio of gel to Gel Solubilization Buffer	Ensure that the correct volume of Gel Solubilization Buffer (L3) is added for every 1 volume of gel used, based on the agarose gel percentage (see page 6).
	Incomplete solubilization of gel piece	Verify that the temperature of water bath or heat block is at 50°C. Cut large gel slices into several pieces to accelerate the gel dissolution. Mix gel slice in the buffer every 3 minutes during the dissolution step.
	DNA fragment is too large	Increase the incubation time for elution to >10 minutes.
	DNA is supercoiled	This kit is not designed to purify supercoiled plasmid DNA from agarose gels.
Low $A_{260/230}$ ratio	Guanidine carryover from the Gel Solubilization Buffer	• Do not get any buffer solution in the cap area of the tube. • Add a second wash step with Wash Buffer (W1): After your first wash with Wash Buffer, followed by centrifugation: 1. Add another 500–700 µL Wash Buffer, containing ethanol. 2. Centrifuge at 12,000 × g. Discard the flow-through and return the column into the Wash Tube. 3. Centrifuge at maximum speed for 2–3 minutes to remove residual Wash Buffer and ethanol.
Downstream enzymatic reactions are inhibited	Residual ethanol in the purified DNA	Traces of ethanol from the Wash Buffer can inhibit downstream enzymatic reactions. To remove Wash Buffer, discard Wash Buffer flow through from the Wash Tube. Place the column into the Wash Tube and centrifuge the column at >12,000 × g for 2–3 minutes to completely dry the column.

Accessory Products

Additional Products

The following products are also available from Invitrogen.

For more details on these products, visit www.invitrogen.com or contact **Technical Support** (page 13).

Product	Quantity	Catalog no.
Qubit® dsDNA Assay Kit, High Sensitivity	500 assays	Q32854
Qubit® dsDNA Assay Kit, Broad-Range	500 assays	Q32853
Qubit® 2.0 Fluorometer	1 each	Q32866

E-Gel® Agarose Gels and DNA Ladders

E-Gel® Agarose Gels are bufferless, pre-cast agarose gels designed for fast, convenient electrophoresis of DNA samples. E-Gel® agarose gels are available in different agarose percentages and well formats for your convenience.

A large variety of DNA ladders is available from Invitrogen for sizing DNA.

For more details on these products, visit www.invitrogen.com or contact **Technical Support** (page 13).

Technical Support

Web Resources

Visit the Invitrogen website at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, SDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog
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SDS

Safety Data Sheets (SDSs) are available at www.invitrogen.com/sds.

Certificate of Analysis

The Certificate of Analysis (CofA) provides detailed quality control information for each product. The CofA is available on our website at www.invitrogen.com/cofa, and is searchable by product lot number, which is printed on each box.

Purchaser Notification

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Purchaser Notification, Continued

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